

In the Specification:

Please amend the specification as shown:

Please delete paragraph [0165] and replace it with the following paragraph:

[0165] In one embodiment the PCR product of step (iii) is produced by a primer pair, of which, one primer has a sequence comprising 5'- YGR CAG GAT ATA T-3' (**SEQ ID NO: 105**) or 5'-CAG GAT ATA TNN NNN KGT AAA C-3' (**SEQ ID NO: 106**).

Please delete paragraphs [0199] to [0201] and replace them with the following paragraphs:

[0199] **Figure [[2]]3.** Gene-free expression cassettes

[0200] **Figure [[3]]2.** Alignment of potato and tobacco invertase inhibitor proteins (**SEQ ID NOS 99, 100, 99 and 101, respectively, in order of appearance**). "St" = *Solanum tuberosum* (potato); "Nt" = *Nicotiana tabacum* (tobacco)

[0201] **Figure 4.** Alignment of trailers associated with various *PPO* genes (**SEQ ID NOS 102-104, respectively, in order of appearance**).

Please delete Table 2 and replace it with the following table:

Table 2. "Border" and "Border-Like" sequences

<i>Agrobacterium</i> T-DNA borders	
TGACAGGATATATTGGCGGGTAAAC (SEQ ID NO.41)	<i>Agrobacterium</i> nopaline strains (RB)
TGGCAGGATATATTGTGGTGTAAC (SEQ ID NO.42)	<i>Agrobacterium</i> nopaline strains (LB)
TGGCAGGATATATACCGTTGTAATT (SEQ ID NO.43)	<i>Agrobacterium</i> octopine strains (RB)
CGGCAGGATATATTCAATTGTAATT (SEQ ID NO.44)	<i>Agrobacterium</i> octopine strains (LB)
TGGTAGGATATATACCGTTGTAATT (SEQ ID NO.45)	LB mutant
TGGCAGGATATATGGTACTGTAATT (SEQ ID NO.46)	LB mutant
YGRYAGGATATATWSNVBKGTAAWY (SEQ ID NO.47)	Border motif
Border-like sequences	
CGGCAGGATATATCCTGATGTAAAT (SEQ ID NO.48)	<i>R. leguminosarum</i>
TGGCAGGAGTTATTCGAGGGTAAAC (SEQ ID NO.49)	<i>T. tengcongensis</i>

ID NO.49)	
TGACAGGATATATCGTGATGTCAAC (SEQ ID NO.50)	<i>Arabidopsis thaliana</i>
GGGAAGTACATATTGGCGGGTAAAC (SEQ ID NO.51)	<i>A. thaliana</i> CHR1v07142002
TGGTAGGATACATTCTGATGTAGAT(<u>SEQ ID NO.107</u>)	Arab. NM114337 (position 1404-1428)
TGACAGGATATATCGTGATGTCAAC(<u>SEQ ID NO.108</u>)	Arab. NM114337 (position 2577-2601)
TGGTAGGATACATTCTGATGTAGTA(<u>SEQ ID NO.109</u>)	Arabidopsis
TTACAGGATATATTAATATGTATGA (SEQ ID NO.52)	<i>Oryza sativa</i> AC078894
TGGCAGGATATCTTGGCATTAAAC(<u>SEQ ID NO.110</u>)	Rice AC037425 (28864-28888)
TGTCAGGATATATATCGATATGAAC(<u>SEQ ID NO.111</u>)	Rice AC097279 (60767-60791)
TGTCAGGATATATATCGATATGAAC(<u>SEQ ID NO.112</u>)	Rice AC097279 (58219-58195)
TAACATGATATATTCCTTGTAAT (SEQ ID NO.53)	Homo sapiens clone HQ0089
TGACAGGATATATGGTAATGTAAAC (SEQ ID NO.54)	potato (left border sequence)*
TGGCAGGATATATACCGATGTAAAC (SEQ ID NO.55)	potato (right border sequence)*

Y= C or T; R= A or G; K= G or T; M= A or C; W= A or T; S= C or G; V= A, C, or G; B= C, G, or T.

The accession numbers for the border-like sequences are: *Oryza sativa* chromosome 10 BAC OSJNBa0096G08 genomic sequence (AC078894.11); *Arabidopsis thaliana* chromosome 3 (NM_114337.1); *Arabidopsis thaliana* chromosome 1 (NM_105664.1); *T. tengcongensis* strain MB4T, section 118 of 244 of the complete genome (AE013091.1); Homo sapiens clone HQ0089 (AF090888.1); *Rhizobium* Clone: rhiz98e12.q1k. *potato left and right border sequences were obtained and isolated according to the presently-described inventive methods.

Please delete paragraph [0321] and replace it with the following paragraph:

[0321] Based on the divergence between P-DNA and T-DNA borders, the elongase amplification system (Life Technologies) was used with the following degenerate primers to isolate a P-DNA from wheat: 5'- GTTTACANHNBNATATATCCTGYCA -3' (Bor-F) (SEQ ID NO. **139**[[56]]), and 5'- TGRCAGGATATATNVNDNTGTAAAC -3' (Bor-R) (SEQ ID NO. 57). The resulting 825-bp fragment is shown in SEQ ID NO.: 2, and was used to replace the T-DNA of a conventional binary vector. The efficacy of this construct can be

tested by inserting an expression cassette for the GUS gene between P-DNA termini, and infecting wheat with an *Agrobacterium* strain carrying the resulting vector.

Please delete paragraph [0325] and replace it with the following paragraph:

[0325] 3. Perform a PCR with a “border” primer that may be degenerate and which anneals to T-DNA border-like sequences, and an “anchor” primer that anneals to the known ligated DNA fragment. Typically, a smear of reaction products is observed upon gel electrophoresis. One example of the border primer is 5'- YGR CAG GAT ATA TNN NNN KGT AAA C -3' (**SEQ ID NO: 113**); an example of anchor primer is 5'- GAC CAC ACC CGT CCT GTG -3' (**SEQ ID NO: 114**). An annealing temperature that was used successfully with these primers is 49⁰C; an extension time of 2.5-minutes may be used for this amplification reaction, although any of the parameters of the PCR amplification reaction may be varied in performing this method.

Please delete paragraph [0330] and replace it with the following paragraph:

[0330] Alternatively, in step 3 of this method an amplification reaction is performed with a first short primer that anneals to the 5' part of border-like sequences such as 5'- YGR CAG GAT ATA T-3' (**SEQ ID NO: 115**) and a second short primer annealing to the ligated DNA fragment with known sequence such as 5'-ATG GCG ACC ACA-3' (**SEQ ID NO: 116**) using relatively high annealing temperatures such as 34⁰C that limit the amount of mismatching. Dilute this DNA about 100-fold, and use 1 µL of the diluted DNA as template for a second PCR with one primer that anneals to at least the middle part and 3' part of border-like sequences such as 5'-CAG GAT ATA TNN NNN KGT AAA C-3' (**SEQ ID NO: 117**), and another primer annealing to the ligated DNA with known sequence that is ideally nested to the short “known DNA” primer described above. One annealing temperature that can be used successfully was 52⁰C.

Please delete paragraph [0340] and replace it with the following paragraph:

[0340] Potato transformations were carried out by infecting stem explants of 4-week-old *in vitro* grown Russet Ranger plantlets with *Agrobacterium* strains according to the following procedure. Ten-fold dilutions of overnight-grown cultures were grown for 5-6 hours, precipitated for 15 minutes at 2,800 RPM, washed with MS liquid medium (Phytotechnology) supplemented with sucrose (3%, pH 5.7), and resuspended in the same

medium to an OD_{600nm} of 0.2. The resuspended cells were then used to infect 0.4-0.6 mm internodal potato segments. Infected stems were incubated for 2 days on co-culture medium (1/10 MS salts, 3% sucrose, pH 5.7) containing 6 g/L agar at 22°C in a Percival growth chamber (16 hrs light) and subsequently transferred to callus induction medium (CIM, MS medium supplemented with 3% sucrose, 2.5 mg/L of zeatin riboside, 0.1 mg/L of naphthalene acetic acid, and 6g/L of agar) containing timentine (150 mg/L) and kanamycin (100 mg/L). After 1 month of culture on CIM, explants were transferred to shoot induction medium (SIM, MS medium supplemented with 3% sucrose, 2.5 mg/L of zeatin riboside, 0.3 mg/L of gibberelic acid GA3, and 6g/L of agar) containing timentine and kanamycin (150 and 100 mg/L respectively). After 3-4 weeks, the number of explants developing transgenic calli and/or shooting was counted. As shown in tobacco, the number of stem explants infected with pSIM108 that showed calli was higher than those in control experiments with the conventional binary vector pBI121 (Table 3). Shoots that subsequently arose from these calli could be grouped into two different classes. The first class of shoots was phenotypically indistinguishable from control shoots transformed with LBA::pBI121. The second class of shoots displayed an IPT phenotype. Shoots of the latter class were stunted in growth, contained only very small leaves, displayed a light-green to yellow color, and were unable to root upon transfer to hormone-free media. To confirm that shoots with an IPT phenotype contained the *IPT* gene stably integrated in their genomes, all shoots were transferred to Magenta boxes containing MS medium supplemented with 3% sucrose and timentine 150 mg/L, allowed to grow for 3 to 4 additional weeks, and used to isolate DNA. This plant DNA served as template in PCR reactions with an oligonucleotide pair designed to anneal to the IPT gene: 5'- GTC CAA CTT GCA CAG GAA AGA C-3' (**SEQ ID NO: 118**), and 5'- CAT GGA TGA AAT ACT CCT GAG C-3' (**SEQ ID NO: 119**). As shown in Table 4, the PCR experiment confirmed a strict correlation between IPT phenotype and presence of the *IPT* gene. The presence of backbone DNA was also examined in plants obtained from a transformation with pBI121. This was done by performing PCR reactions on DNA isolated from the transformation events with the 'pBI121 backbone primers': 5'- CCGTGTAAGTGAAGTGCAGTTGCCATG-3' (SEQ ID NO. 64), and 5'- CATCGGCCTCACTCATGAGCAGATTG-3' (SEQ ID NO. 65). Amplification of a 0.7 kbp band is indicative for backbone integration. By comparing the data presented in Table 4, it can be concluded that backbone integration frequencies are similar for P-DNA vectors and T-DNA vectors.

Please delete paragraph [0354] and replace it with the following paragraph:

[0354] As an alternative to the leader-based approach, expression cassettes that contained both a sense and antisense copy of the trailer sequence associated with *R1* were generated. This trailer was obtained by performing a reverse transcription polymerase chain reaction (RT-PCR) on total RNA isolated from microtubers of the potato cultivar Russet Ranger. Complementary DNA was generated using the Omniscript RT Kit (Qiagen, CA) and then used as a template for a PCR reaction with Hot start DNA polymerase (Qiagen, CA) with the gene-specific reverse primer R1-1 (5'-GTTCAGACAAGACCACAGATGTGA-3' **(SEQ ID NO: 120)**). Sequence analysis of the amplified DNA fragment, cloned in pGEM-T demonstrated that the trailer associated with R1 consists of 333 basepairs (SEQ ID NO.: 16). The sense and antisense copies of the trailer were separated by either the Ubi intron or the GBSS spacer- and sandwiched between GBSS promoter and Ubi3 terminator (Figure 3; SEQ ID NOs.: 17-18). Similar versions with the larger GBSS promoter are shown in Figure 3 (SEQ ID NOs.: 19-20).

Please delete Table 1 and replace it with the following table:

Table 1. Potentially expressed uncharacterized peptides in antisense potato lines

<i>Gene (size of fragment used)</i>	<i>Predicted peptides encoded by ORFs in reverse-complemented DNA</i>
<i>R1</i> (1.9-kb)	MSSTSNVGQD CLAEVTISYQ WVGRVINYNF FLIIHWYTVV EASTGITFQI FPIGIRSEDD RSFYEKADRF A WVT (SEQ ID NO: 121)
	MSSESTFSKT PNGRATDVGI PTEEGTFPFR YAILRDLAPT ISLVNSSADI A (SEQ ID NO: 122)
	MSEGVGFKSK ILPSFAWRS NILGSKHVAK QTFPFLARTE TCERTSGMSG VIRATAPSGI SSSPLTDFAT KIVGFS (SEQ ID NO: 123)
GLTP (1-kb)	VCSPALKADK SKSADGTCVD HSRRLIVVLV LYPGMGTSYA TAFISSPPIQ YLFPSDPVET FP (SEQ ID NO: 124)
	MLGSLVLPKS PENRKQAVPN PHFQEQHLVP EKPHFLDCGQ GFSKLPQMHQ (SEQ ID NO: 125)
	MVNFLTQGIV DMETAFGSPK MGGFGKEQFG ACVSRSEMDE SGIGAVMVEQ VCSICSRHFV LSMQI (SEQ ID NO: 126)
GHTP (0.9-kb)	MLEGSMWPWN QESMKRAFLN HHFLMLHLFP AQRPPQAADP VCLKHQHMHG GCLSFQLHLS KLAPGDTPLI SSMFALD (SEQ ID NO: 127)
	MKLCSSIILS IIKQKQVEIL RACFGFPETK TISVFSSVSW NWHICKSL (SEQ ID NO: 128)
	MTKKPDRKDN IMPYNFPGTK FLQPIFRNFF LPSLCDKLLK KSI SVPQAIT PCWKVQCGHG IKKA (SEQ ID NO: 129)

PPO (1.8-kb)	TILKLDLHTF NGHFFTASFW NQSHRNSIFI FQSNILQQFS YRQLESNTGN MISITSMNM RQASITPCKL RLIKLIHHS LVHVQKHIEP YIVPIIRYF IECQYLLLLI FLLCCP (SEQ ID NO: 130)
	MKGKEKPREM NLQFFTTNFV STVAISTMNI SLLFKAKRVK GVFIKFPST RSQILGYVL LIRMSRGAD AEFSHRREL V VRNTIDLIGY RRATTVYYIN TFFYMGSTTR LEIRRWYRCS SR (SEQ ID NO: 131)
	MEWALARNRI PFFYCPNSLR TSHGKGYDFH RRKRIQSSTN LYLLNPFPSR QLISIHSTSC PHWHGGSKKS DLNRVSRNYP CLHRFFDEV C HRSRCEPEYE GCFQ (SEQ ID NO: 132)
SBE A (1.2-kb)	MNNITHSPIL IPFLEQLNPF ISNCHMQPIV KANTPILNGN TKCRHSANIF TNGNCIWEKP MNKIVDQHQI HNSIHSCS KVFLVVPSES HR (SEQ ID NO: 133)
	MKFRYPSPN PIVTSLIILC NAIPRSINDV DGLSRAIKSY ISLSISQNAI VLSPTRA (SEQ ID NO: 134)
SBE B (2.6-kb)	MVNIMTSSSM ATKFPSITVQ CNSVLPWQVT SNFIPFVCVL WVEVEYKYQV TTFKHNNLII IHAAYYLF (SEQ ID NO: 135)
	MAKLVTHEIE VPLSSQGHCE KMDHLVKRNS SINNRRSICQ ARHARIHLFV H (SEQ ID NO: 136)
	MFETKLNSGV VWNDWLTVNI RNSNTPNTKL VLLHHVVRTV PSIEIANNFV FLSSRSPFTI DYATIFPVES KF (SEQ ID NO: 137)
	MLYTSLYISY LSNSMLLP SW TNLHHSYSLN NLSTYLGLPL PGGNQNNQFLP QKQAGQGPAY QKHLRQ (SEQ ID NO: 138)

Please delete the paragraphs on page 171, lines 27-36 and replace them with the following paragraphs:

SEQ ID NO. [[96]]**140**

GTCCATGATGTCTTCAGGGTGGTAGCATTGACTGATTGCATCATAGTTTTTTTTTT
TTTTTTAAGTATTTCTCTATGCATATTATTAGTATCCAATAAATTTACTGGTTGTT
GTACATAGAAAAAGTGCATTTGCATGTATGTGTTTCTCTGAAATTTTCCCCAGTTT
TTGGTGCTTTGCCTTTGGAGCCAAGTCTCTATATGTAATAAGAAAATAAGAACA
ATCACATATATCAAATATTA

SEQ ID NO. [[97]]**141**

ACGAACCTTGATCGCGTTGAAAGATTTGAACGCTACTTGGTCATCCACAT
AGAGCTTCTTGACGTATCTGGCAATATTGCATCAGTCTTGGCGGAATTTTCATGTG
ACAAAAGGTTTGCAATTCTTTCCACTATTAGTAGTGCAACGATATACGCAGAGAT
GAAGTGCTGAACAAACATATGTAAAATCGATGAATTTATGTCGAATGCTGGGAC
GGGCTTCAGCAGGTTTTGCTTAGT